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G1B BAD BAG BAX BCB B105 B307 B525

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(58) Field of search
UK CL (Edition K) G1B BAD BAG
INT CL[5] G01N
Online databases: WPI, BIOTECH(DIALOG)

(54) Immunodiffusion or immunoelectrophoresis methods

(57) Invention relates to improvements in the detection of the presence of complementary agents in samples, particularly extractable nuclear antigens, by monitoring the formation of a precipitate or light scattering matrix.

The method and apparatus are particularly concerned with the control of the application of the samples to a test body (11), normally a gel. By applying the samples to the surface of a test body and removing excess liquid, the permeation of particulate matter into the test body can be substantially eliminated. In conventional methods such matter can smother the results of a test. The careful control of the regions to which the samples are applied has further useful advantages in speed and improved detectability when the effective separation of the samples are reduced by interlocking the sample application regions. A mask (10) is proposed as the most advantageous method of controlling the regions of application.

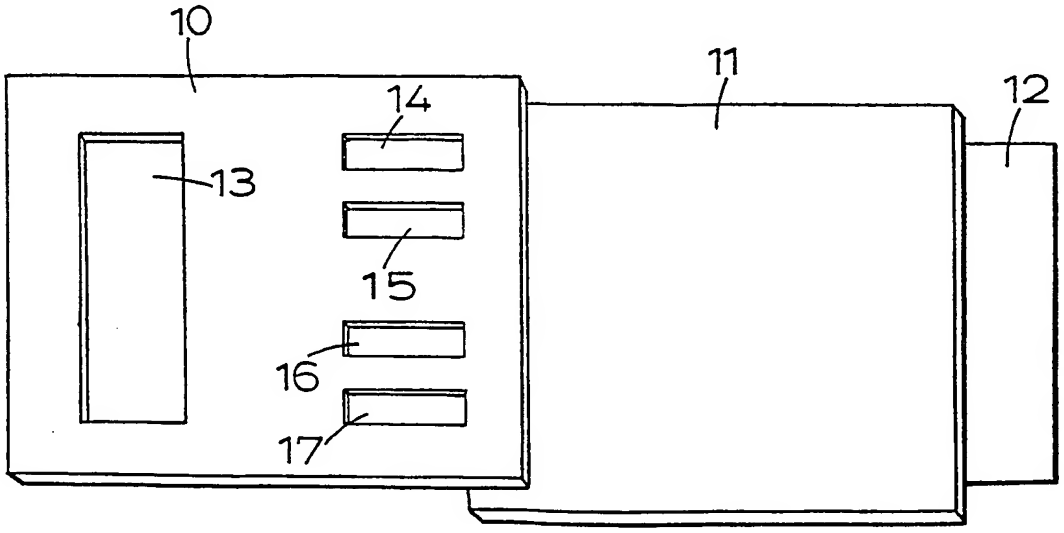

FIG.4.

At least one drawing originally filed was informal and the print reproduced here is taken from a later filed formal copy.

The claims were filed later than the filing date within the period prescribed by Rule 25(1) of the Patents Rules 1990.

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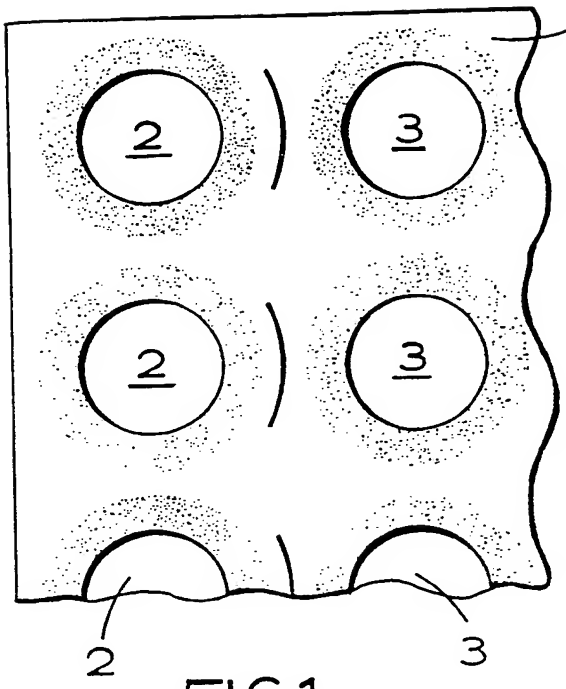

FIG.1.

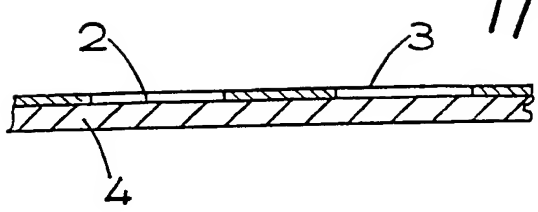

FIG.2.

PRIOR ART

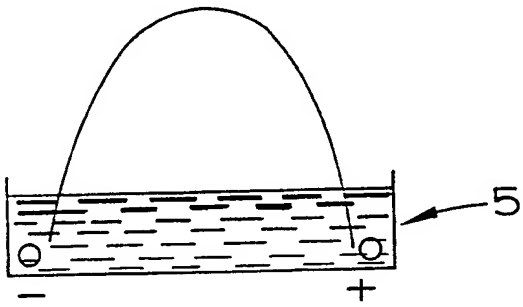

FIG.3.

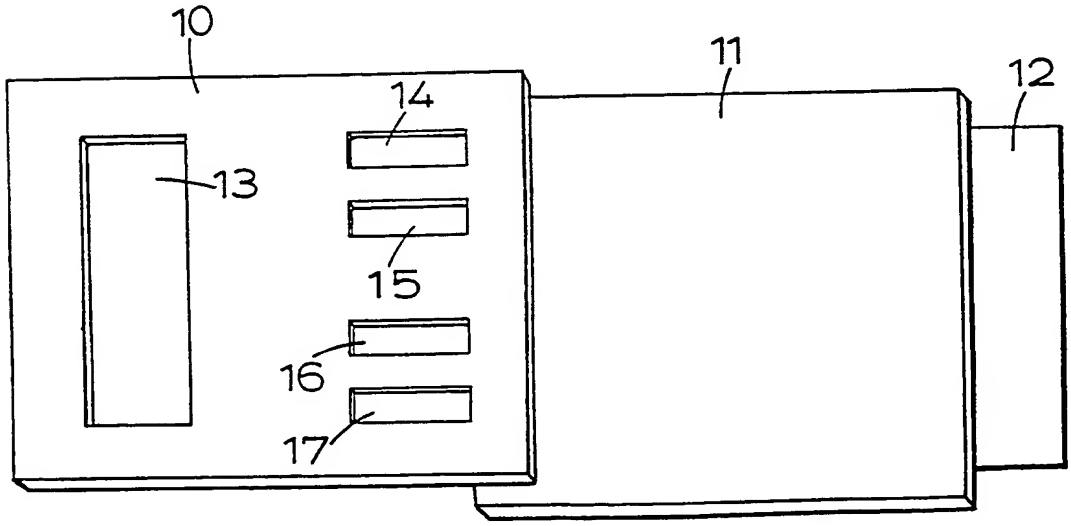

FIG.4.

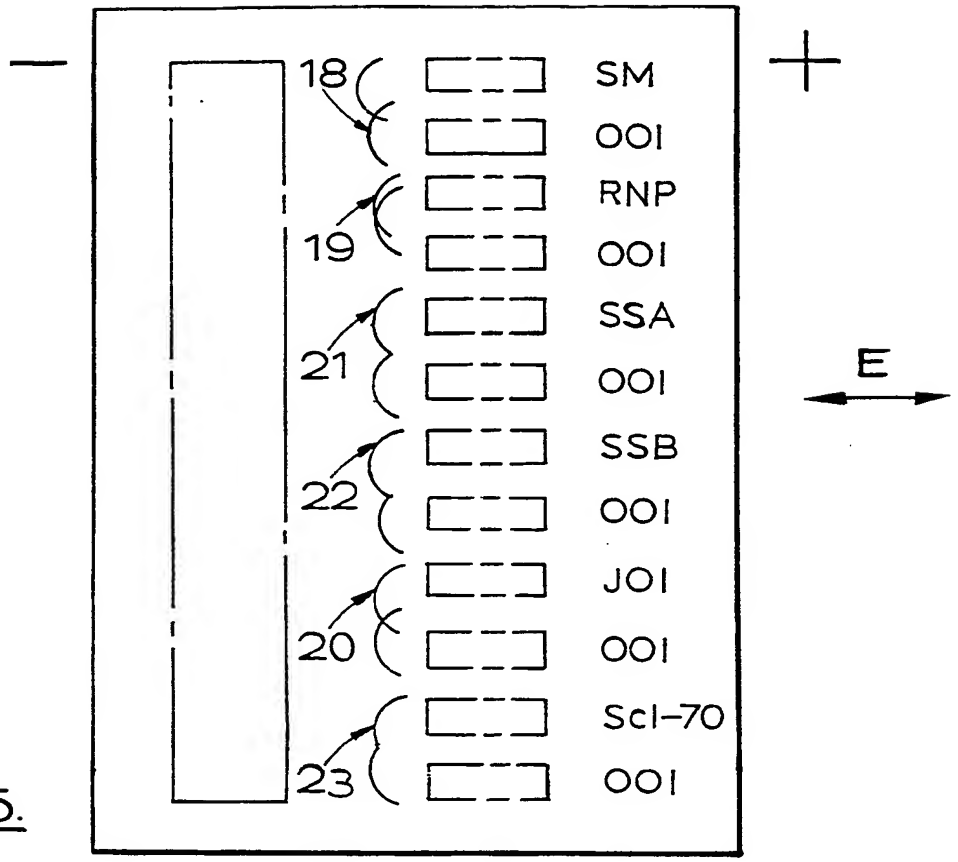

FIG.5.

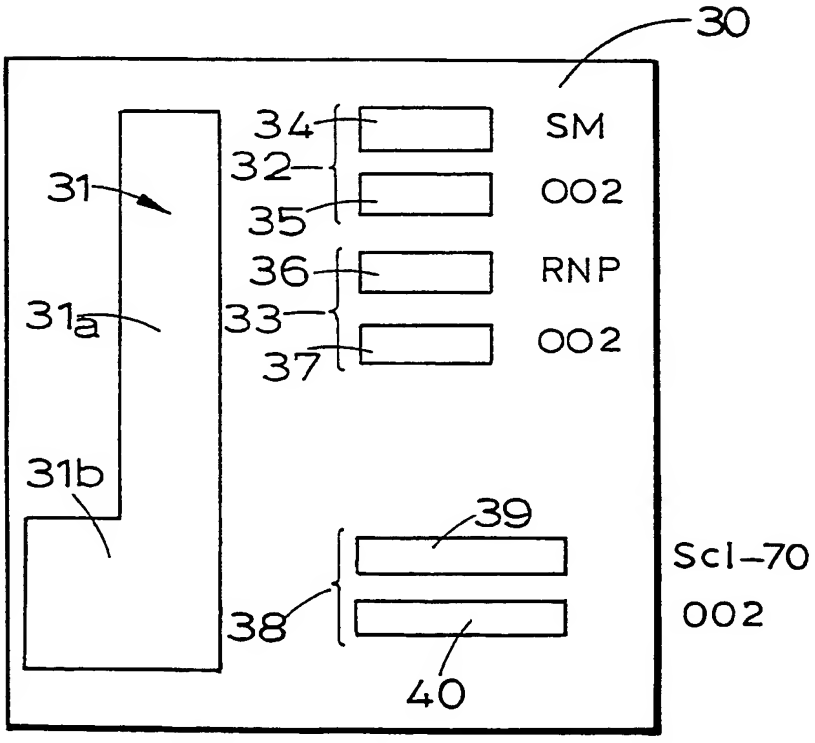

FIG.6.

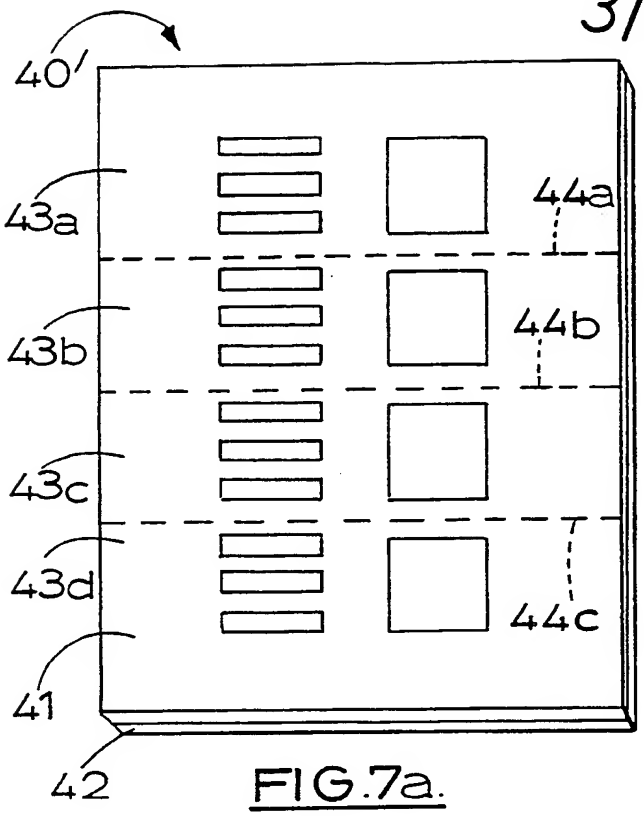

FIG.7a.

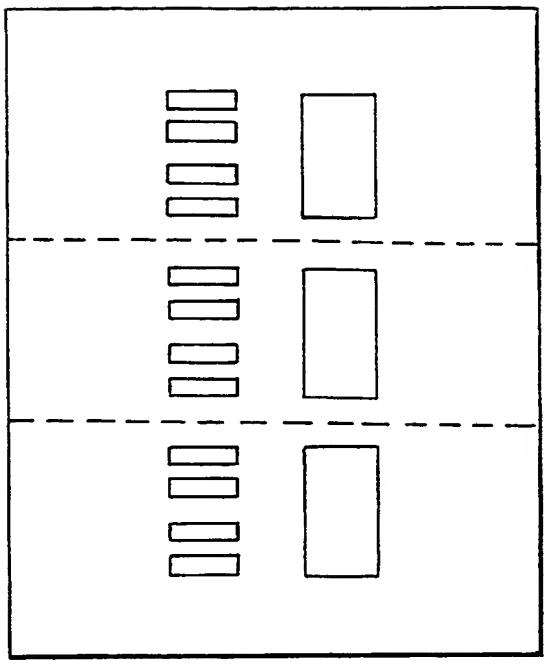

FIG.7b.

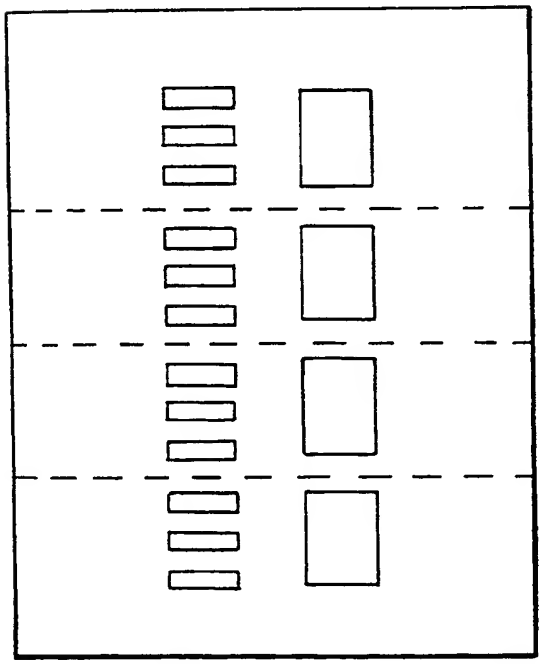

FIG.7c.

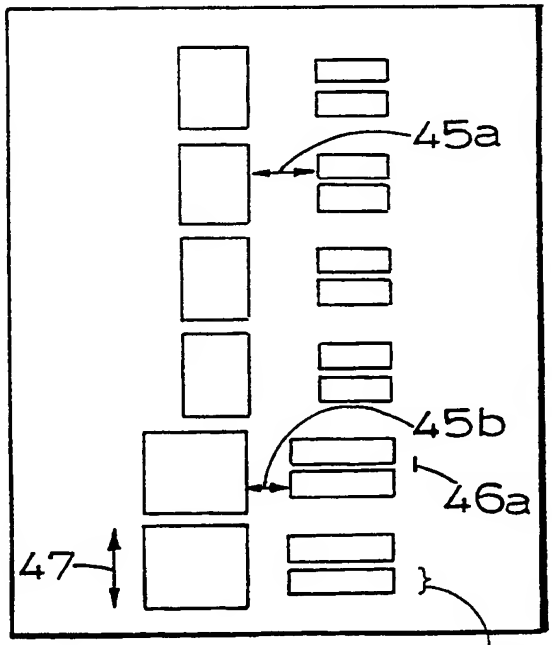

FIG.7d

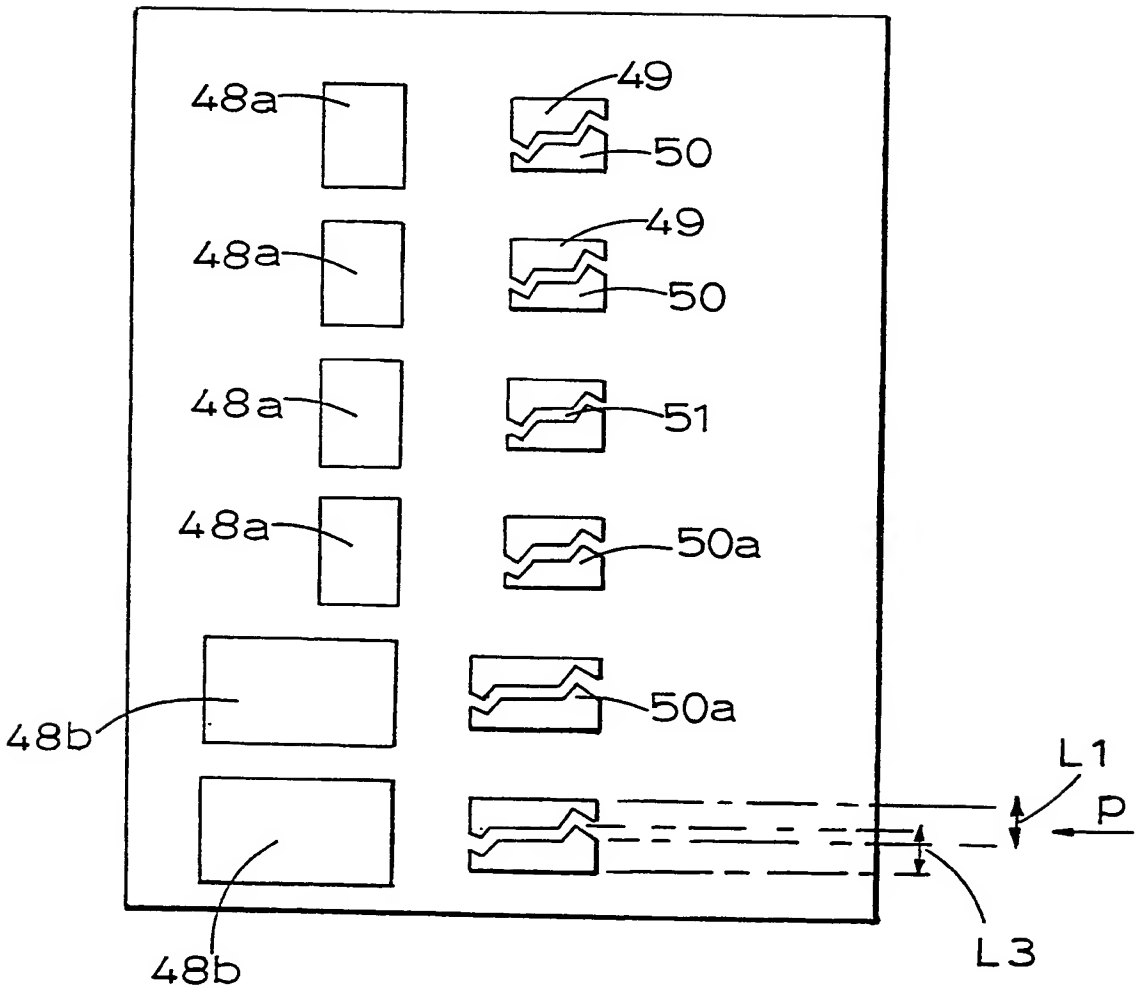

FIG. 8.

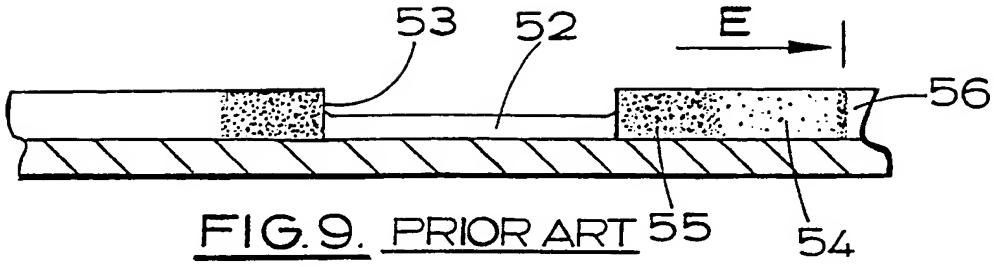

FIG.9. PRIOR ART

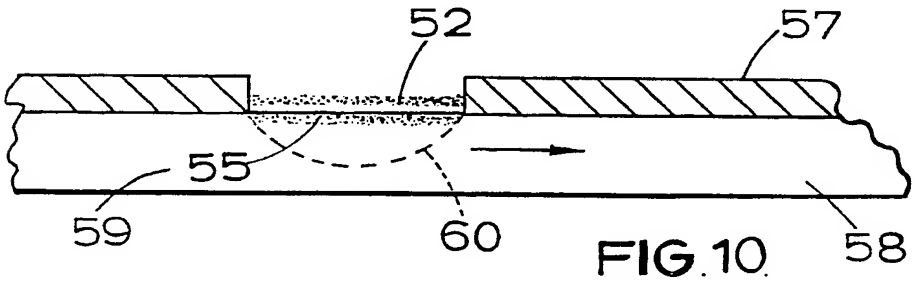

FIG.10

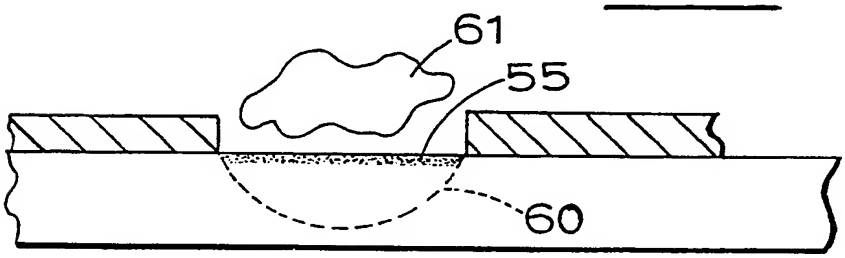

FIG.11.

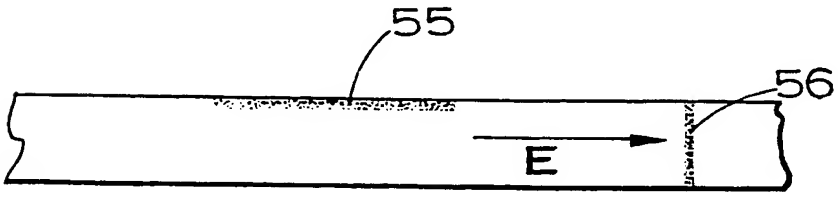

FIG.12.

DETECTING ANTIBODIES TO EXTRACTABLE NUCLEAR ANTIGENS AND OTHER SUBSTANCES

This invention relates especially, but not necessarily exclusively, to a method and apparatus for detecting and/or identifying antibodies to Extractable Nuclear Antigens (ENA).

The invention arose from work connected with detecting and identifying antibodies to ENA. It is known that the presence of certain antinuclear antibodies in serum can be indicative of certain diseases or conditions, for example scleroderma.

In fact anti-nuclear antibodies react with more than one nuclear constituent including DNA, histones, non-histone proteins and protein-RNA complexes. It is normally insufficient to report a significant titre of ANA without performing further tests to identify the detailed specificities of the autoantibodies which have important clinical associations. The generic term, extractable nuclear antigen (ENA), is used for many of these antigens as they are saline soluble macromolecules; it is a misnomer as they are extracted from both the nuclei and cytoplasm of various cells.

As purified antigens are not readily available yet, routine anti-ENA detection and identification depends on immunodiffusion methods. Characterised reference sera are used to establish the identity of lines of precipitation produced by positive test sera.

There are many known ways of trying to identify lines of identity which are produced when a test serum precipates or matrixes with a reference serum. One example of such techniques is Ouchterlony double

diffusion, but the method which is generally considered to be the best is counter-immuno-electrophoresis (CIE).

In the known CIE methods a thick sheet (eg 1 or 2mm) of agarose gel is prepared and a pair of rows of circular through-holes are cut in the sheet using a template. One row of holes defines reservoirs for test sera containing test antigens and the other complementary row of holes defines reservoirs for samples to be tested. The user places a quantity of test and sample liquids in their respective holes in the agarose gel and places the sheet into an electrophoresis tank where electrophoresis occurs for about 80 minutes. The sheet is left overnight and then examined for lines of precipitation where the respective pairs of antibodies and antigens have reacted. If a sample liquid exhibits a positive response it can then be type-tested to see what antibodies are present.

It is an aim of the present invention to provide a new way of testing for and/or identifying antibodies to ENA.

According to a first aspect of the invention we provide a method of detecting the presence of the first of a pair of complementary precipitate or matrix-forming agents in a sample in which a first liquid containing the first agent is applied to a surface region of a test body through which the first agent can migrate; allowing the first agent to migrate through the test body until it is incorporated in a precipitate or light scattering matrix, the light-scattering properties of the matrix differing from those of the test body, and using a change in the light-scattering resulting from the formation of the

matrix to detect the presence of the matrix and consequently the presence of said first agent in the sample.

Preferably the first liquid is applied to the surface only of the test body.

Preferably the first liquid is allowed to remain on the surface of the test body for a time so as to allow the first agent to migrate into the thickness of the gel, and then surplus, or any liquid remaining on the surface of the body, is removed.

Preferably the method is a method of detecting and/or identifying antibodies to ENA, or ENA.

The method preferably comprises applying the first liquid comprising a sample to the test body, and applying a second liquid containing the second agent of said pair of complementary matrix-forming agents to the surface region of the test body.

The method may also comprise applying a third, reference, liquid containing a known reference first agent to the surface region of the test body and comparing the reaction of said first and third liquids with said second liquid.

The method preferably comprises using a mask having one or more apertures to defines areas of the test body which receive the first, or second, or when provided, the third, liquid. The mask is preferably in contact with the test body when liquid is introduced into its apertures. The mask is preferably removed after excess liquid has been removed from the surface of the test body, but it may be removed before then.

The method preferably includes using electrophoresis to cause the agents to migrate towards each other.

The test body is preferably a body of gel having a flat upper surface, and most preferably a sheet of gel.

The method preferably comprises using a mask to control the application of a volume of the first liquid on a first region of the surface of the gel, and to control the application of a volume of the second liquid at a second region of the surface of the gel which is spaced from the first region, waiting a time such that relatively small things in the liquids, including said first and second agents, migrate into the thickness of the gel, removing any remaining liquid from the apertures of the mask before substantial quantities of relatively large particulate matter which may be present in either, or both, of the liquids can migrate deeply into the gel; and removing the mask.

Electrophoresis and/or staining may follow.

The method also preferably includes the step of ensuring that at least some of the apertures in the mask defining the first or second regions of the surface of the gel are elongate in the direction away from the other of said second or first region.

The method may comprise arranging for there to be a pair of associated, but spaced apart, first regions and a common second region.

According to a second aspect of the invention we provide a method of increasing the sensitivity of tests for detecting the presence of the first of a pair of

complementary precipitate or matrix-forming agents in a sample comprising applying to a first region of a test body a first liquid containing the first agent and applying to the test body at a second region a second liquid containing the second agent, in which the first and second regions are spaced apart, and in which at least one of the regions is elongate in the direction away from the other region.

It is advantageous to decrease the time required to perform a test of the kind involving the mutual contact of three liquids, for example a test in accordance with the first or second aspects of the invention. This may be done by reducing the distance the first and third liquids must travel to contact one another, because of the initial position of the first and third liquids, relative to one another, within the test body.

The initial position of the first and third liquids is determined by the method by which the liquids are introduced to the test body.

The first and third liquids are introduced in a way which maintains them as substantially separate liquids, that is, spaced by a strip having a minimum width within the test body, prior to the start of the test. The arrangement of the first and third liquids may be such that when viewed along a line of travel, and projected onto another line perpendicular to said line of travel, the projected distance between the part of each liquid closest to the other liquid, measured in the direction perpendicular to the direction of the line of travel, is less than the minimum strip width. This provides the decrease in the time required to perform the test and may comprise a third aspect of the invention.

The first and third liquids may be arranged in such a way that a part of each liquid overlaps with the other liquid with respect to the direction perpendicular to travel.

According to a fourth aspect the invention comprises a method of increasing the speed of reaction of a test of the kind involving the mutual contact of three liquids, the liquids migrating in a test body; the method comprising applying the first and third liquids to the test body at first and third regions and applying the second liquid to the test body at a second region spaced from the first and thirds regions in a direction hereinafter referred to as the primary direction, the first region having a line length defined by the length and position of the projection in the primary direction of the first region onto a line perpendicular to the primary direction, and the second region having a line length defined by the length and position of the projection in the primary direction of the third region onto said line perpendicular to the primary direction, and wherein the line length of the first region touches or overlaps the line length of the third region, or wherein the line lengths of the first and third regions are closer together on said line perpendicular to the primary direction than any portions of the first region is to any portion of the third region measured along the direction of said line perpendicular to the primary direction.

Preferably the line lengths of the first and third portions overlap.

The first region may have a projection, overlap, or offset, portion extending towards the third region. The offset portion preferably contributes that part of

the line length of the first region which is closest to the nearest part of the line length of the third region, on which overlaps the line length of the third region.

The third region may also have a projection, overlap, or offset, portion.

The offset portions of the first and third regions preferably interlink the first and third regions.

The first and/or third region may have a recess portion adapted to accommodate a projecting portion of the third and/or first region.

According to another aspect of the invention we provide a mask for use in the first, second, or third aspects of the invention, the mask having a plurality of apertures comprising at least one first aperture adapted to receive a first liquid, and at least one second aperture adapted to receive a second liquid.

The first aperture is preferably elongate in the direction away from the second aperture.

Preferably there is a third aperture, adjacent the first aperture and spaced from the first aperture by the same amount. The second aperture may extend in a direction generally parallel to that of the relative spacing of the first and third apertures to effectively form a slot extending for substantially the length of the row of two or more of the first and third apertures.

The first and third apertures are preferably separated by a spacing, such that the adjacent edges of

the first and third apertures are at least a minimum spacing apart.

The adjacent edges may be arranged such that the distance between the point on each aperture closest to the other aperture, measured in a direction perpendicular to the spacing between the first and second apertures, is less than the minimum spacing.

In the most preferred arrangement the perpendicular distance between the closest points may be reduced to zero, or less than zero, in that the apertures overlap in the perpendicular direction.

A plurality of spaced pairs of first and third apertures may be provided.

The second aperture may have a non-uniform width in the direction transverse to the direction from the second aperture to the, or the row of, first aperture(s). The width of the second aperture may have a stepped increase. When there are a plurality of pairs of first and third apertures the elongate width of some of the pairs (in the direction away from the second aperture) may be different from that of other pairs.

According to a further aspect of the invention we provide a kit comprising a mask in accordance with the previous aspect of the invention and a test body, preferably a sheet of gel, for use in accordance with the first and/or second aspect of the invention.

The kit may have means for removing liquid from the apertures of the mask. These means may comprise blotting means, such as a sheet of paper.

The kit preferably includes instructions to use it in accordance with the first and/or second aspect of the invention.

The kit may have the mask and the test body provided initially as a combined unit, the mask being removable later.

The combined unit may have discrete operational regions which can be separated from each other so as to enable different portions of the unit to be used at different times.

Embodiments of the invention will now be described by way of example only with reference to the accompanying drawings of which:-

Figures 1 and 2 show a prior art agarose gel sheet having wells for test and sample liquids, and shows a typical test result;

Figure 3 shows an alternative prior art agarose gel sheet in an electrophoresis tank;

Figure 4 shows a mask and gel sheet in accordance with the present invention;

Figure 5 shows a gel sheet after electrophoresis, and shows a typical test result using the method of the present invention;

Figures 6 and 7a to 7d show alternative masks;

Figure 8 shows a further alternative mask; and

Figures 9 to 12 illustrate a theory of why the present invention works.

Figures 1 and 2 show the known way of using CIE to test for antibodies to ENA. An agarose gel sheet 1 has a row of sample liquid holes or wells 2 punched through it and a corresponding row of test liquid holes or wells 3 punched through it. The sheet 1 is supported on a petri dish 4 (or other support as will be described later). The user places a substantial quantity (about 50µl) of sample liquid containing serum from a subject to be tested into the holes 2, and a corresponding volume of test liquid containing reference antigens (ENA) in the holes 3. Different subjects can be tested against a cocktail of ENA's (screen testing) or a subject can be tested against a range of specific ENA's in different wells 3 (type testing).

The user places the sheet 1 in an electrophoresis tank for one to two hours and studies the results of the precipitation reaction between any antibodies which may have been present in the sample and the test ENA's.

Figure 1 shows lines 6 of precipitation, and dark "halos" surrounding the wells 2 and 3. The dark halos can in some cases extend out radially to such a distance that they make it difficult, or impossible, to identify precipitation lines, especially if there are several precipitation lines (indicating different antibodies). Furthermore, when the concentration of antibodies in the test liquid is low it is usual to use an increased volume of test liquid to compensate and provide a roughly comparable molar number of antibodies in the sample. This can make worse the problem of the

dark halos of precipitation obscuring the precipitation lines.

It is now believed that the dark halos are caused by particulate matter and other light absorbing/reflecting molecules in the sample liquid (and in the test liquid) migrating into interstices in the body of the gel. These particles congregate together and effectively become stuck in the gel (or at least do not migrate anywhere near as quickly as "free" antibodies and antigens). It will be appreciated that the clumping together of the particulate matter involves a different mechanism from antibody/antigen precipitation, but that the clumps of particulate matter are large enough to effect the optical properties of the gel and form the "halo", or cloud around the wells.

Figure 3 shows schematically another conventional way of conducting electrophoresis in which the sheet 1 of agarose gel is supported by a flexible backing sheet and is bent as it is put into the electrophoresis tank. This method of electrophoresis is difficult to do with conventional ENA testing since the pools of liquid on the wells 2 and 3 tend to run out of the wells. This is especially so if the diameter of the wells is large so that they have a long length in the inclined direction.

Figure 4 illustrates the contents of a kit which can be used to perform a new method of ENA testing. The kit comprises a mask 10, a sheet of agarose gel 11 (for example of the kind known as "gelbond"), and a sheet of blotting material 12.

The mask 10 has a large rectangular hole 13 formed in it and four rectangular holes 14 to 17 which are elongated in the direction transverse to the hole 13. The holes 14, 15 and 16, 17 are provided in two pairs.

The user rests the mask 10, which is of plastics material or the like, on the agarose sheet 11 and places a volume (Xμl) of a cocktail of a wide range of reference antigens in the hole 13, which thus comprises an antigen hole. A volume (Yμl) of a first know reference antibody liquid (say SM) is placed in hole 14 and a similar volume of sample liquid derived from a subject to be tested is placed in hole 15. Thus hole 14 is a reference hole, and hole 15 a sample hole. Similarly, Uμl of a second known reference antibody liquid (say RNP) is placed in hole 16 and a second volume of sample liquid from the same subject placed in hole 17. The user then waits for a relatively short time to enable the antibodies and antigens to diffuse into the gel layer, but not long enough for very much of the particulate protein matter that the antigen liquid, reference antibody liquid, and sample liquid will contain, to diffuse it to the gel. The particulate material cannot diffuse into the gel layer because it is too big to do so. A suitable time to wait might be about 15 minutes.

The user then blots the mask using the blotting material 12 to soak up any liquid which may still be present in the holes 13 to 17.

The mask is then removed and the, non-holed, agarose sheet with particles of antigen cocktail, reference antibody, and sample liquid diffused into it, is placed in an electrophoresis tank for 1 hour, or more.

It may be possible to see lines of identity between the reference antibody and the sample of a pair of patches. Alternatively, it may be necessary to stain the sheet to highlight lines of precipitation at antibody/antigen reactions. This general kind of test is known in the art as a "typing test".

The lines of precipitation are easier to see because there is less problem associated with particulate matter in the liquids.

Figure 5 shows the results of a typing test for patient 001 against the antibodies SM,RNP,SSA,SSB,JO1, and Scl-70. The positions of the holes in the mask used to create the original particles of liquid in the gel are shown in chain dotted line.

Non-identity is illustrated at 18,19 and 20 where precipitation lines form adjacent reference antibody - sample pairs cross. Identity of antibody is shown at 21,22, and 23 where the precipitation lines meet without crossing. The direction of electrophoresis is shown as arrow E.

Figure 6 shows a mask 30 suitable for use with subject samples in which at least some of the antibodies of interest are likely to be present at lower than convenient concentrations. The mask 30 has an L-shaped hole 31 having a relatively narrow region 31a and a relatively wide region 31b for a cocktail of antigens, and two pairs 32 and 33 of holes 34 to 37 for SM, sample 002,RNP and sample 002, respectively. The concentration of SM and RNP in the sample 002 is expected to be in a range which is easily worked with. The mask also has a pair 38 of holes 39 and 40 for antibody Scl-70 and a sample 002

respectively. It is expected that the concentration of SCl-70 in the sample 002 will be particularly low - low enough such that if used with the mask of Figure 5 the results relating to Scl-70 may be inconclusive.

The elongate length of the holes 39 and 40 is larger in the direction parallel to the directions of electrophoresis than that of the holes 34 to 37. Thus the holes 39 and 40 have a greater area than that of the holes 34 to 37. The patches of liquid which move into the gel in the regions of the holes 39 and 40 are larger, and hence more antibodies than would otherwise be the case are present in the patches resulting from holes 39 and 40. This gives a better (darker) precipitation line.

Figure 7a shows another mask and sheet of agarose gel combination unit 40' having four testing regions. The mask is referenced as number 41 and the gel sheet as number 42. The mask and sheet combination is supplied to the user as a combined unit. The combination unit 40' has four similar operational regions 43a to 43d connected by connecting regions 44a to 44c. The user can cut the unit at one or more of the connecting regions. This enables the user to use less than a whole unit 40', and to save the other portion of it for use later. Thus, the mask has been separated into regions so that the user does not necessarily have to perform complete tests. The mask 41 is suitable for testing for JO1 and Scl-70.

Figure 7b shows another mask with three regions, and its holes provided in pairs within the regions.

Figure 7c shows a screening mask.

Figure 7d shows a typing mask in which the distance 45a is about 10mm, the distance 45b, about 6mm, the distance 46a about 1mm, the distance 46b about 4mm, and the distance 47 about 13mm.

The user places appropriate liquids in the holes of the mask, waits a while, blots or otherwise removes excess liquid from the holes of the mask, removes the mask from the gel sheet, electrophorises the sheet, and stains it if necessary.

Although the provision of reference and sample holes in the mask is preferably in pairs (so that it is easy to look for lines of identity) it will be appreciated that we could provide then in groups of three (positive control, sample, and negative control).

Figure 8 shows another typing mask. The mask is provided with holes 48a, 48b for the cocktail of reference antigens. The holes 48b are larger to test for antibodies which are likely to be present at lower concentrations as disclosed above. Holes 49 are provided for the various control samples and holes 50 are provided for the samples from the subject to be tested.

The control and sample holes are arranged in interlocking pairs separated by a spacing strip 51.

The mask is used in the same way as the method disclosed above, however, the design of the mask apertures is such that the time taken to perform the test is greatly reduced.

Figure 8 also shows the lengths of the holes 49 and 50 projected in the direction of a primary

direction (referenced P) onto a line perpendicular to the primary direction, these lengths being considered to be the line lengths L1 and L3 of the holes 49 and 50. It will be noted that the line length of hole 49 overlaps that of hole 50. The "teeth" on the holes need not cause the line lengths to overlap, but could simply cause the line lengths of the holes to be closer together than they would otherwise be if the holes had flat straight sides. Thus holes with non-straight sides could be another way of looking at this feature.

During the time in the electrophorisis tank the antigens introduced into the gel through holes 48a, 48b move to the right, as seen in Figure 8, as the antigens are negatively charged. The antibodies introduced via holes 49, 50 in the mask migrate from right to left as they are positively charged. The diagnostic lines for the test are formed by the interaction of the three liquids within the gel. To obtain a line of identity, or determine non-identity, the sample and known antibody, from holes 50 and 49 respectively, must come into contact. The further these two liquids have to travel to come into contact the longer the test takes. By overlapping the apertures to an extent in the direction perpendicular to the direction of migration the control and unknown sample come into direct contact quicker as the portion 50a, at least, of the sample migrates along the same path as the control. This is much quicker than relying on the tendency of the samples to radiate during migration and so come into contact that way.

So far we have described typing tests, but it will be appreciated that the invention can be used to carry

out screening tests using the same test logic as the known ENA tests, but using the new apparatus and method.

Similarly, although the tests discussed have been for antibodies present in patients' bodies, the test can be reversed to detect and identify antigens using a cocktail of antibodies and reference antigens. The invention may well have applications in more general multi-component precipitation or matrix-forming test systems, which need not necessarily be for ENA's, or related to them.

Figures 9 to 12 illustrate a theory as to why the present invention improves on the known techniques, but it will be understood that we do not warrant the accuracy of the theory.

The prior art is shown in Figure 9. A liquid 52 sits in a well 53 of the gel for many hours. The liquid contains relatively small antigens or antibodies 54 and relatively large particulate matter 55. The large particulate matter (usually proteinaceous) is drawn into the interstices of the gel by diffusion and eventually particles are attracted together, or simply get stuck together in the same or nearby interstices. The aggregation of the particles makes them visible, at least when stained. (Alternatively the particles may not need to be in conglomerations for them to affect the optical properties of the gel.) The particulate matter enters the gel over the whole depth of the side walls of the well 53 and is present throughout the thickness of the gel. This can make electrophoresis slower. Line 56 shown in Figure 9 represents the eventual precipitation line of antibody/antigen. It will be noted that it has

a depth - that is to say extends for the thickness of the gel.

Figure 10 shows a mask 57 on top of a gel 58 with liquid 52 in the hole in the mask. The liquid and relatively small antibodies or antigens diffuse or otherwise migrate into the gel fairly quickly (in, say, 10 to 30 minutes) and initially reach a relatively deep portion 59 of the gel. Hypothetical boundary 60 illustrates schematically the soaking up of the liquid and antibodies/antigens. The larger particulate matter 55 cannot move so quickly in the gel and so stays near to the surface of the gel during the relatively short time that the liquid is left on the gel.

Figure 11 shows the mopping up of excess liquid from the hole in the mask. This may be done with a blotting motion, using a blotting member (schematically shown as reference number 61), or it may be wiped or washed off.

When the electric field of electrophoresis is applied the antibodies/antigens in the deeper regions of the gel can move unhindered by particulate matter (because particulate matter is only present at the surface region). Furthermore, since a body of liquid containing a particulate matter is not left on the gel during electrophoresis (or for any time long enough for too much particulate matter to enter the gel) there is no so much particulate matter present, and it does not move so far.

The above theory explains why the dark halos of the prior art are not skewed by the electric field (because the particulate matter is either uncharged, or

so big that its charge to mass ratio is too small to produce a noticeable effect), and why the new method has better results.

The clarity of the precipitation lines 56 of the new method can be far better than that of the known methods of anti-ENA testing. This allows us to test for more dilute concentrations of antibodies. The invention may also be quicker since the particulate matter may not interfere so much in the migration of the antibodies/antigens.

In the present invention, large particles do not penetrate the gel, and large volumes of liquids can be applied whereas in the known method where liquid is held in holes the liquid easily spills out. Furthermore, large holes in the gel sheet interrupt the current flow during electrophoresis and so distort the results.

CLAIMS

1. A method of detecting the presence of the first of a pair of complementary precipitate or matrix-forming agents in a sample in which a first liquid containing the first agent is applied to a surface region of a test body through which the first agent can migrate; allowing the first agent to migrate through the test body until it is incorporated in a precipitate or light scattering matrix, the light-scattering properties of the matrix differing from those of the test body, and using a change in the light scattering resulting from the formation of the matrix or precipitate to detect the presence of the matrix and consequently the presence of said first agent in the sample.

2. A method according to claim 1 in which the first liquid is applied to the surface only of the test body.

3. A method according to claim 2 in which the first liquid is allowed to remain on the surface of the test body for a time so as to allow the first agent to migrate into the thickness of the gel, and then removing surplus liquid, or any liquid remaining on the surface of the body.

4. A method according to any preceding claim which comprises applying the first liquid comprising a sample to the test body, and applying a second liquid containing the second agent of said pair of complementary matrix-forming agents to the surface region of the test body.

5. A method according to claim 4 which further comprises applying a third, reference, liquid containing a known reference first agent to the surface region of the test body and comparing the reaction of said first and third liquids with said second liquid.

6. A method according to any preceding claim which comprises using a mask having one or more apertures to define areas of the test body which receive the first, or second, or when provided, the third, liquid.

7. A method according to claim 6 in which the mask is in contact with the test body when liquid is introduced into its apertures.

8. A method according to claim 6 or claim 7 in which the excess liquid is removed from the apertures of the mask after a predetermined time.

9. A method according to claim 8 in which the mask is removed after excess liquid has been removed from the surface of the test body.

10. A method according to any one of claims 6 to 9 which includes the step of ensuring that at least some of the apertures in the mask defining the first or second regions of the surface of the test body are elongate in the direction away from the other of said second or first region.

11. A method according to any preceding claim which further comprises arranging for there to be a pair of associated, but spaced apart, first regions and a common second region.

12. A method according to any preceding claim which comprises using a mask to control the application of a volume of the first liquid on a first region of the surface of the gel, and to control the application of a volume of the second liquid at a second region of the surface of the gel which is spaced from the first region, waiting a time such that said first and second agents migrate into the thickness of the gel, and removing any remaining liquid from the apertures of the mask before substantial quantities of relatively large particulate matter which may be present in either, or both, of the liquids can migrate deeply into the gel; and removing the mask.

13. A method according to any preceding claim which further comprises using electrophoresis to cause the agents to migrate towards each other.

14. A method of increasing the sensitivity of the tests for detecting the presence of the first of a pair of complementary precipitate or matrix-forming agents in a sample comprising applying to a first region of a test body a first liquid containing the first agent and applying to the test body at a second region a second liquid containing the second agent, in which the first and second regions are spaced apart, and in which at least one of the regions is elongate in the direction away from the other region.

15. A method of detecting the presence of the first of a pair of complementary precipitates or matrix-forming agents in a sample substantially as described herein with reference to any of Figures 4 to 12 of the accompanying drawings.

16. A method of decreasing the time required to perform a test of the kind involving the mutual contact of three liquids, the method comprising introducing to a test body first and third liquids in a way which maintains them as substantially separate liquids, that is, spaced by a strip having a minimum width within the test body, prior to the start of the test. The arrangement of the first and third liquids being such that when viewed along a line of travel, and projected onto another line perpendicular to said line of travel, the projected distance between the part of each liquid closest to the other liquid, measured in the direction perpendicular to the direction of the line of travel, is less than the minimum strip width.

17. A method according to claim 16 in which the first and third liquids are arranged in such a way that a part of each liquid overlaps with the other liquid with respect to the direction perpendicular to travel.

18. A method of increasing the speed of reaction of a test of the kind involving the mutual contact of three liquids, the liquids migrating in a test body; the method comprising applying the first and third liquids to the test body at first and third regions and applying the second liquid to the test body at a second region spaced from the first and third regions in a direction hereinafter referred to as the primary direction, the first region having a line length defined by the length and position of the projection in the primary direction of the first region onto a line perpendicular to the primary direction, and the third region having a line length defined by the length and position of the projection in the primary direction of the third region onto said line perpendicular to the primary direction, and wherein the line length of the

first region touches or overlaps the line length of the third region, or wherein the line lengths of the first and third regions are closer together on said line perpendicular to the primary direction than any portions of the first region is to any portion of the third region measured along the direction of said line perpendicular to the primary direction.

19. A method according to claim 18 in which the line lengths of the first and third portions overlap.

20. A method according to claim 18 or claim 19 in which the first region has a projection, overlap, or offset, portion extending towards the third region.

21. A method according to claim 20 in which the offset portion contributes that part of the line length of the first region which is closest to the nearest part of the line length of the third region, or which overlaps the line length of the third region.

22. A method according to any one of claims 18 to 21 in which the third region has a projection, overlap, or offset, portion.

23. A method according to any one of claims 18 to 22 in which the first and third regions have offset portions which interlink the first and third regions.

24. A method according to claim 23 in which the first and/or third region has a recess portion adapted to accommodate a projecting portion of the third and/or first region.

25. A method of increasing the speed of reaction of a test liquid of the kind involving the mutual contact of

three liquids substantially as described herein with reference to Figure 8 of the accompanying drawings.

26. A mask adapted for use in the method of any preceding claim, the mask having a plurality of apertures comprising at least one first aperture adapted to receive a first liquid, and at least one second aperture adapted to receive a second liquid.

27. A mask according to claim 26 in which the first aperture is preferably elongate in the direction away from the second aperture.

28. A mask according to claim 26 or claim 27 in which there is a third aperture, adjacent the first aperture and spaced from the first aperture by the same amount.

29. A mask according to claim 28 in which the second aperture extends in a direction generally parallel to that of the relative spacing of the first and third apertures to effectively form a slot extending for substantially the length of the row of two or more of the first and third apertures.

30. A mask according to claim 28 or claim 29 in which the first and third apertures are separated by a spacing such that the adjacent edges of the first and third apertures are at least a minimum spacing apart.

31. A mask according to claim 30 in which the adjacent edges are arranged such that the distance between the point on each aperture closest to the other aperture, measured in a direction perpendicular to the spacing between the first and second apertures, is less than the minimum spacing.

32. A mask according to claim 31 in which the perpendicular distance between the closest points is reduced to zero, or less than zero, in that the apertures overlap in the perpendicular direction.

33. A mask according to any one of claims 26 to 32 in which a plurality of spaced pairs of first and third apertures are provided.

34. A mask according to any one of claims 26 to 33 in which the second aperture has a non-uniform width in the direction transverse to the direction from the second aperture to the, or the row of, first aperture (s).

35. A mask according to any one of claims 26 to 34 in which the width of the second aperture has a stepped increase.

36. A mask according to claim 34 or claim 35 in which there are a plurality of pairs of first and third apertures and the elongate width of some (or at least one) of the pairs (in the direction away form the second aperture) is different from that of other pairs.

37. A mask substantially as described herein with reference to Figure 4; or Figure 5; or Figure 6; or Figure 7a; or Figure 7b; or Figure 7c; or Figure 7d; or Figure 8 of the accompanying drawings.

38. A kit comprising a mask in accordance with any one of claims 26 to 37 and a test body, preferably a sheet of gel, for use in accordance with any one of claims 1 to 25.

39. A kit according to claim 38 which has means for removing liquid from the apertures of the mask.

40. A kit according to claim 39 in which the mask and the test body are provided initially as a combined unit, the mask being removable later.

41. A kit according to claim 40 in which the combined unit has discrete operational regions which can be separated from each other so as to enable different portions of the unit to be used at different times.

42. A kit substantially as described herein with reference to any of Figures 4 to 12.

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Relevant Technical fields

(i) UK Cl (Edition K) G1B (BAD; BAG)

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(ii) Int Cl (Edition)

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Databases (see over)

(i) UK Patent Office

(ii) ONLINE DATABASES:WPI, BIOTECH (DIALOG)

Date of Search
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Documents considered relevant following a search in respect of claims 1-13,15,25,37,42

Category (see over)	Identity of document and relevant passages	Relevant to claim(s)
X	GB 1030474 (J G FEINBERG) - see particularly page 2 lines 46-94	1,2,4-7, 10,11,13, 15,37,42
X	US 3482943 (MILES LABORATORY) - see particluarly column 2 lines 7-19, column 4 line 65 - column 5 line 5	1,2,4-7, 10,11,13, 15,37,42
X	US 3622484 (L P CAWLEY) - see particularly figures 3,4	1,4,5,11, 13

Category	Identity of document and relevant passages	Relevant to claim(s.

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